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Heritability of kinship pheromone in the beaver: how is information about relatedness coded?*

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Abstract We attempted to estimate the narrow-sense heritability of single compounds and whole profile of the anal gland secretion (AGS) with the regression method using several sets of relatives in the beaver *Castor canadensis*. We used GC (gas chromatography) and GC-MS (mass spectrometry) to characterize and quantify chemical compounds in beaver AGS. We found that the heritability of single compounds seemed to be low, whereas the heritability of AGS profile appeared to be moderate. We conclude that many compounds might be involved in the coding of genetic relatedness, which was through a combination of analog and digital coding using many compounds [*Acta Zoologica Sinica* 50 (4): 504 - 510, 2004].

Key words Heritability, kinship, Pheromone, Information coding, Beaver, *Castor canadensis*

河狸亲缘信息素的遗传力：遗传关系的信息是怎样编码的？*

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摘 要 本研究利用几种亲缘关系对河狸（*Castor canadensis*）肛腺分泌物中的单个化合物和其整体组合的狭义遗传力进行了估算。使用气相色谱和质谱对河狸肛腺化合物进行定性和定量分析，发现了单个化合物的遗传力很低，但化合物的整体组合却显示有适度的遗传力。因此，我们认为亲缘关系的编码可能涉及多种化合物，并以数量和数字编码并用的方式进行［动物学报 50（4）：504 - 510，2004］。

关键词 遗传力 亲缘信息素 信息编码 河狸

Heritability has long been reckoned as one of the essential prerequisites for evolution to happen since Darwin (1859). More specifically, natural selection is a depletive process for additive variation (Fisher, 1930), and heritability sets the upper limit for natural selection to work (Falconer, 1989). Thus, estimating heritability is a crucial step in elucidating how animal behavior evolves. Investigating heritability of communication signals can provide crucial knowledge about the additive variation in the signals and allow us to infer the evolutionary processes that have happened in the past. These insights have been illustrated in measuring heritability or repeatability of the acoustic and chemical signals in insects (*e. g.*, Hager and Teale, 1994; Hedrick, 1994). However, little such information is available for vertebrates. Therefore, studies of heritability of chemical signals in vertebrates are important for a better understanding of the evolutionary process of vertebrate chemical communication systems.

In an earlier study in the beaver *Castor canadensis*, we showed that related individuals are more similar to one another than to unrelated individuals in anal gland secretion (AGS) and that AGS compounds are

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highly stable and repeatable for each individual over at least several years (Sun and Müller-Schwarze, 1998a). This implies that the AGS profile (collective feature of types and abundances of major compounds) may be heritable (Hartl and Clark, 1989). It, however, does not provide any information about how much additive genetic variation is present in the profile. Nor does it reveal information about the heritability of individual compounds. As for how information about relatedness is coded, two possibilities: either a few or many compounds are used (Sun and Müller-Schwarze, 1998b). Yet, until now we have not been able to determine which option is more likely. The answer to this question is critical for revealing the coding and detection systems for chemical communication in the beaver. It will also shed light on mammalian chemical communication in general.

In the case of the beaver, if levels of individual compounds are heritable, then it is likely that beavers may use a few compounds for communicating information about relatedness, and the mode of information coding and transmission is mainly analog (quantitative coding using the same signal components; also known as graded signals) rather than digital (qualitative coding with different signal components). Analog transmission requires that receivers should have a high resolution in their sensory system to differentiate minute differences in the relative quantities of these AGS compounds. Conversely, if individual compounds are not heritable, digital coding and transmission are more likely for information coding about genetic relatedness in the beaver.

Because heritability intuitively indicates that closely related individuals are more similar than distantly related individuals for the trait measured, we thus attempted to study the heritability of beaver AGS compounds to examine the two competing hypothesis for the coding of genetic relatedness using the kinship pheromone: digital versus analog. Specifically, we predicted that if AGS compounds are individually heritable, analog coding is plausible. If AGS compounds are not individually heritable, digital coding seems to be more likely.

1 Marerials and methods

1.1 Sample collection

We trapped beavers in Allegany State Park, New York, from 1992 to 1995 using Hancock live traps baited with aspen *Populus tremuloides* twigs. Captured beavers were anaesthetized by injecting a mixture of xylazine and ketamine (Schulte, 1993). We sexed beavers by the color and viscosity of AGS (Schulte et al., 1995) and validated the results by palpating for the presence or absence of the os penis. We assigned beavers to four age classes (kit, one-year-old, two-year-old, and adult) based on body weight and size (Schulte, 1993), and trapping records. Both ears of each individual were tagged with a unique color combination of anodized aluminum ear tags (National Band & Tag Co., Kentucky). We milked secretion samples from the anal glands into 10 ml glass vials lined with Teflon inside the lids and allowed two hours for beavers to fully recover from the drug before release. We quickly transported the samples into the field laboratory, added methylene chloride (5∶1 in volume) into each sample and stored it in a freezer (- 20 ℃). These samples were later transported to Syracuse, New York, in a cooler box packed with dry ice or ice and then stored in a freezer (- 20 ℃) until analysis. Tests using gas chromatography did not show detectable decomposition in these samples during our study.

For each AGS sample, 1 ml of AGS-methylene chloride solution was used (0.2 ml pure AGS material). We removed the methylene chloride by distillation and redissolved the residue in ether (3 ml). We washed the organic solution with brine (3 × 3 ml), dried it over sodium sulphate, and stored it in a freezer at - 20 ℃.

1.2 Compound characterization

We separated and characterized AGS compounds in ether extract using gas chromatography combined with mass spectrometry (GC-MS) (Hewlett-Packard HP5890 Series Ⅱ-HP5971 Series). An HP-1 30 m × 0.25 mm fused silica capillary column (Hewlett-Packard Co.) was used. We injected 2 μl of the AGS-ether solution and used the following temperature program: 130 ℃ (0.5 min), 2 ℃/min, and 230 ℃ (10 min). We used the first 50 minutes as collection time. Few compounds were detected after 50 minutes, and peaks were often not stable even if they did.

To measure heritability of AGS compounds, we characterized each compound in an AGS sample using its GC retention time (Sun and Müller-Schwarze, 1998b for representative GC spectra with same retention times) backed up by mass spectrum (Sun 1996, for mass spectra) so that different compounds with similar GC retention times could be discerned. The integration function in HP G1034B Software for MS ChemStation (Hewlett-Packard Co.) was used to obtain the relative abundance of each compound. We used the signal/noise ratio of 2 as the threshold so that all major peaks were included, whereas excessively small peaks were excluded. Approximately equal numbers of AGS compounds were obtained from each individual for either sex (20 - 25 from a male sample and 35 - 40 from a female sample). For convenience, we use the word 'profile' to refer to the compounds and their relative abundances occurring in

the 50 minutes collection period.

1.3 Data analysis

Family genealogy for each site was constructed based on intensive trapping and observation data. Since it is unlikely that beavers can control the absolute amount of secretion material when they deposit AGS, the relative amount of compounds in AGS is more likely to be biologically meaningful than the absolute amount. Therefore, we converted the absolute amount of each compound into the relative amount so that the proportion of all compounds in a sample added up to one.

We used several regression coefficients to estimate the narrow-sense heritability for each AGS compound: father - son, father - daughter, mother - son, mother - daughter, brother - brother, and brother - sister. We also attempted to use the mid-parent - offspring estimate, but we did not present the results due to small sample sizes ($n < 10$). We used full-sibs only for comparisons with heritabilities estimated from other sets of relatives, and the dominance effect and environmental covariance were not removed for full-sib estimates due to inadequate sample sizes. Maternal effects were not excluded when a mother - offspring set was used for heritability estimation. The regression slopes from between-sex estimations (father - daughter, mother - son, and brother - sister) were adjusted by the ratio of phenotypic standard deviations of males and females (Falconer 1973). To increase the reliability of regression analysis, a compound selected for heritability estimation should meet the following two conditions: (1) it was present in at least 50 % of individuals used for heritability estimation, and (2) there were at least 10 pairs of individuals, each pair showing non-zero quantity for this compound.

To estimate the heritability of the whole AGS profile, we used principal component analysis as a data reduction method to capture the main features of AGS profiles with a few orthogonal canonical variables (Johnson and Wichern, 1992). We initially selected those compounds that were present in more than 50 % of individuals and excluded those rare compounds that may not play an important role to represent features in AGS profile. The PROC PRINCOMP procedure (SAS Institute, Inc. 1990) was used to perform all calculations. The regression coefficients of each individual with the first three principal components were used as three independent measurements for heritability estimation. We estimated these heritabilities using exactly the same relative sets as we did with single compounds. Because of the presence of sex-specific compounds, males and females were analyzed separately for within-sex (father-son, mother-daughter, and brother-brother) heritability estimation. To estimate between-sex heritability, we used the compounds present in both sexes after the preliminary screening. The slopes in between-sex regression analyses were adjusted by the same method as we did with single compounds. The significance level for all tests was set at 0.05.

2 Results

2.1 Heritability of single AGS compounds

The heritability estimates from the nine relative sets are presented in Table 1. Most heritability estimates were not significantly different from zero. Only one out of 93 (1.06 %) estimates (including repeated estimation for the same compounds) was significantly different from zero. This was not significantly different from results obtained by chance (5 %) alone (binomial test, two-tailed, $P = 0.788$). Although 44 (26.19 %) heritability estimates were considered large (≥ 0.5, including those larger than 1.0), none of them could be cross-validated using other relative sets when available. Therefore, there was little evidence to show that individual compounds were heritable. Some heritability estimates seemed to fall out of the reasonable range between 0 and 1, but taking the standard errors into consideration, none of them significantly deviated from this range. In addition, there was no difference in obtaining estimation values above or below zero (two-tailed binomial test with normal approximation, $n = 168$, $Z = 0.258$, $P = 0.795$), indicating that heritabilities of individual AGS compounds may overall be close to zero.

2.2 Heritability of AGS profile

Heritability estimates of the AGS profile represented by the first three principal components are shown in Table 2. The first three principal components accounted for 69.3 %, 57.1 % and 66.1 % of the original information for the three data sets (22 compounds from males, 31 from females, and 25 from both sexes, respectively). For the first principal component, three out of the nine heritability estimates were larger than 0.5, one was close to 0.5, and three out of the remaining five were moderate. The mother^3/daughter estimate was significantly larger than 0 ($t_9 = 3.392$, one-tailed, $P = 0.004$). For the second principal component, three out of the nine estimates were larger than 0.5, and the brother-brother estimate was significantly larger than 0 ($t_{26} = 2.809$, one-tailed, $P = 0.005$). For the third principal component, all but one of the nine estimates were moderate. The estimates from the relative sets between brothers and between father and daughter were significantly larger than 0 ($t_{26} = 2.985$ and $t_{13} = 3.194$, $P = 0.003$ and 0.004, respectively). Although some estimates were beyond the reasonable range of heritability (between 0 and 1), none of these

Table 1 Heritabilities of anal gland secretion compounds

CPD	RT[a]	Heritability estimate													
		FS			FD			MS			BB			BS		
		h^2	*SE*	*n*	h^2	*SE*	*n*	h^2	*SE*	*n*	h^2	*SE*	*n*	h^2	*SE*	*n*
1	11.160													0.385	0.645	23
2	23.039	0.821	0.531	18							-0.447	0.587	23	-0.009	0.050	30
3	23.781	-0.782	0.659	19	-1.031	0.480	14	-1.097	0.974	15	0.253	0.572	24	0.061	0.227	38
4	26.560	-0.112	0.599	17							-1.087	0.598	16			
5	28.038	-0.613	0.872	16							-0.052	0.447	21			
6	28.280										-0.348	0.463	13			
7	28.880	0.299	0.748	19				-1.222	0.952	15	0.965	0.653	24	0.094	0.065	37
8	29.113	-0.120	0.644	19	-0.222	0.497	14	1.716	1.383	15	0.559	0.695	24	0.293	0.159	30
9	29.280	-0.204	0.787	11							-0.512	0.470	15			
10	29.411	-0.333	0.561	16	-0.702	0.413	13	1.467	3.059	15	-0.397	0.399	23	0.072	0.096	30
11	29.773	-0.219	0.802	19	-0.555	0.387	14	-0.970	0.599	15	0.742	0.671	24			
12	30.380	-1.812	1.253	12	1.006	0.859	12	-0.243	0.725	12	-0.997	0.492	16	-0.020	0.260	31
13	35.358	0.829	0.668	19							-0.038	0.469	23			
14	43.299	1.706	0.912	19	0.788	0.448	14	1.066	0.769	15	-0.435	0.507	24	-0.082	0.116	31
15	43.880							0.640	1.287	10				-1.273	0.694	31
16	44.424										-1.304	0.616	12			
17	44.594	0.694	1.968	13							0.248	1.018	19			
18	45.576	0.850	1.423	18				0.328	0.854	13	-0.593	0.495	24			
19	45.878													-1.049	0.581	21
20	45.898	1.186	1.329	11							-0.775	0.534	16			
21	46.523	1.972	0.942	18				0.399	1.059	13	-0.428	0.512	24	0.104	0.243	22
22	47.377	0.567	0.775	16							-0.030	0.541	21			
23	47.849										-1.304	0.486	11	-0.231	0.217	26
24	47.879	0.074	2.380	17							-0.893	0.458	21			
25	48.327	1.026	0.756	19	0.956 *	0.402	14	1.568	1.099	15	-0.269	0.457	24	-0.074	0.136	31
26	48.572	1.752 *	0.740	19							-0.325	0.480	24			
27	48.935	-0.157	0.264	18							-1.055	0.564	16			
28	49.000	-0.028	1.358	17							-0.852	0.436	21			
29	49.290							1.038	1.878	14	-1.182	0.599	16	-0.090	0.176	30
30	49.327							0.716	1.619	14	-1.151	0.743	19	-0.122	0.179	30
31	49.847	-0.443	1.190	15							0.000	0.000	11			
32	49.860							-1.757	1.454	12				-0.246	0.184	22
33	50.000	-1.030	0.552	19	-0.563	0.516	14	0.535	0.773	15	-0.758	0.524	24	0.005	0.248	31

a The mass spectra for all compounds listed here are available in Sun(1996).

CPD: Compound number. RT: GC retention time. FS: Father-son. FD: Father-daughter. MS: Mother-son. BB: Brother-brother. BS: Brother-sister. h^2: Narrow sense heritability. SE: Standard error. *n*: Sample size. * $P < 0.05$.

Table 2 Heritability of anal gland secretion profile represented by the first three principal components

Relative set for estimation	Principal component 1			Principal component 2			Principal component 3				
	h^{2a}	SE^b	IL^c	h^2	SE	IL	h^2	SE	IL	n^d	CIL^e
Father-son	0.760	0.554	0.429	0.158	0.360	0.170	0.383	0.590	0.094	22	0.693
Mother-son	0.212	1.272	0.423	0.825	1.502	0.155	0.269	0.630	0.084	13	0.661
Father-daughter	0.009	0.272	0.423	0.780	0.763	0.155	1.084 *	0.339	0.084	14	0.661
Mother-daughter	0.969 *	0.286	0.321	0.241	0.240	0.148	0.407	0.616	0.102	10	0.571
Brother-brother	0.239	0.428	0.429	0.900 *	0.320	0.170	0.829 *	0.278	0.094	27	0.693
Brother-sister	0.035	0.076	0.423	0.106	0.202	0.155	0.428	0.213	0.084	33	0.661

a: Narrow-sense heritability. b: Standard error. c: Information load of the principal component. d: Sample size for regression analysis. e: Cumulative information load of the three principal components. * $P < 0.05$.

Numbers of compounds include: 22 for males, 31 for females and 25 for males and females combined.

deviations were significant. The heritability estimates for the three principal components were reasonably consistent among the nine different estimation methods. More importantly, the heritability of AGS profile was moderate.

3 Discussion

During the previous 11 years of intensive trapping and observation, all family members for the colonies used in our study were individually identified, and their family lineages constructed. Beavers are socially monogamous (Bradt, 1938; Novak, 1977; Svendsen, 1989; Müller-Schwarze and Sun, 2003; Sun, 2003), despite occasional exceptions (Wheatley, 1993; Sun, 2003). Additionally, beaver ponds at our study area are frozen in the mating season between January and February (Hodgdon and Hunt, 1953; Bergerud and Müller, 1977; Olson and Hubert, 1994). Therefore, beavers were physically constrained from leaving their own colonies for extra-mating opportunities (Sun, 2003). Hence, the family lineages constructed based on live-trapping and intensive observation should be reliable.

A common problem in measuring heritability is large standard errors due to small sample sizes, making precise estimation difficult (Falconer, 1989). This appears to be true for most of the heritability estimates in our study. Precise estimations of heritability often require hundreds of families (Hartl and Clark, 1989), which are usually difficulty to obtain for large animals. Despite small sample sizes, three facts are noticeable in our study. First, regression coefficients estimated from sample sizes that were not too small (> 20) failed to show relatively better estimates than those from smaller samples for individual compounds. This may indicate that these compounds were lowly or not heritable. Second, results obtained from different sets of relatives were not consistent. This is contrary to the heritability estimates of whole AGS profiles represented by principal components. Finally, the probability of obtaining significant heritability estimates was not different from chance alone, and the overall heritability for individual AGS compounds was not different from zero. These results all point to the same conclusion: there may be little or no additive variation in individual compounds. Due to small sample sizes, however, these results may only show a possible trend that needs further verification.

Lack of heritability may be due to intense selection pressure. This is well-documented in traits closely related to fitness. These traits are sensitive to selection, and additive variation has been depleted in the past. Although it is possible that little additive variation, the intensity of selection on individual AGS compounds is currently not estimable, nor is it known why selection should be strong for these traits. If selection did play an important role in depleting the additive variation in individual AGS compounds, we should expect a low heritability in the whole AGS profile as well. This is not so in our study. Therefore, past selection seems unlikely to be responsible for the low additive variation in AGS compounds.

Linkage disequilibrium is another plausible reason for the low heritability of individual AGS compounds. The most likely physical correspondent of linkage disequilibrium is that the genes controlling the expression of a quantitative trait are on the same chromosome. Linked genes cannot recombine freely so that the effect is not additive (Scharloo, 1987). This is true if the genes controlling AGS profile formation are a tightly linked multi-gene family like the MHC genes, which have attracted much attention in the study of chemical communication in rodents and humans (Yamazaki et al., 1983, 1992; Beauchamp et al., 1985; Ferstl et al., 1992; Maxson, 1992; Pearse-Pratt et al., 1992; Sommerville et al., 1995; Wobst et al., 1995).

While few individual compounds are heritable, the AGS profile as a whole appeared to be moderately heritable. This may be the reason why the first three

principal components consistently gave positive (with a few exceptions) heritability estimates from different sets of relatives. It is consistent with the finding that related individuals are more similar in their AGS profiles than non-related individuals (Sun and Müller-Schwarze, 1998a). Evidence shows that AGS profiles do not change with environmental conditions (Sun and Müller-Schwarze, 1998a), and thus environmental covariance may be small when full sibs were used for estimating heritability. Since larger sample sizes are required for reducing the standard errors (Arnold, 1994), our heritability estimates may not provide information about the exact values. Nonetheless, the relatively high consistency of the results using different relative sets still indicate that the AGS profile was heritable. This finding adds further evidence to a positive association between genetic relatedness and AGS profile similarity (Sun and Müller-Schwarze, 1998a). Because compounds were not heritable independently, individual compounds may not be important in coding for genetic relatedness. So, coding for information about genetic relatedness should involve many AGS compounds.

In an earlier study, we found that either 2 - 3 or a large number of compounds may be used to code for genetic relatedness, but we failed to determine which option was more likely at that time (Sun and Müller-Schwarze, 1998b). Because little additive variation existed in single AGS compounds, the combination of two or three compounds was unlikely to be highly heritable, and thus insufficient to code for genetic relatedness. Therefore, coding for genetic relatedness should involve many compounds. However, because the heritability of the AGS profile was modest, analog coding by many compounds is partially, but not entirely, responsible for coding for genetic relatedness. So, digital coding may also be involved in the coding. That is, genetic relatedness in the beaver was based on a combination of both analog and digital coding. There is also a possibility, albeit small, that trace or rare compounds that were excluded from our analysis, may play certain roles in information coding. This needs further investigation.

Detection of information coded in analog signals, such as genetic relatedness, requires a highly sensitive sensory (detection) system. Since signaling systems evolve much faster than signal detecting systems (Ryan, 1990; Ryan et al., 1990; Ryan and Rand, 1993, 1995), selection should be stronger on signalers. One potential advantage of using many different compounds in signal coding is that more digital components can be added to the coding system to increase the reliability of communication. This may be the reason why beavers tend to use the whole AGS profile, or at least many compounds in it, to code for information about genetic relatedness. Yet, how to measure the heritability of digital signals is a new challenge for the future.

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